REMARKS

This paper is filed in Response to the Office Action mailed December 10, 2010. Claims 27 to 32, 34 to 43 and 48 to 54 are under consideration. New claims 55 to 59, directed to the elected invention have been added. Accordingly, claims 27 to 32, 34 to 43 and 48 to 59 are under consideration.

Applicants respectfully request an interview with the Examiner of sufficient duration to discuss all grounds for rejection that may remain upon consideration of this paper and the accompanying documents submitted herewith.

The Amendment to the Specification

The specification has been amended to correct an informality. In particular, the specification has been amended to correct the date for the deposited biological material, the SAM-6.10 antibody producing hybridoma. As evidenced by the accompanying Revised Statement under 37 C.F.R. §1.804(b) executed by Dr. Frank Hensel, Applicants had possession of the hybridoma that produces SAM-6.10 antibody and the SAM-6.10 antibody heavy and light chain variable region sequences referenced in the application, for example, at page 13, lines 8-9, at the time the application was filed.

Thus, the amendment was made to address an informality no new matter has been added. Accordingly, entry thereof is respectfully requested.

The Claim Amendments

The claim amendments are supported throughout the specification. In particular, the amendment to claims 27 and 43 to delete reference to a specific light chain (V_L) variable region sequence (SEQ ID NO:1) is supported, for example, by originally filed claims 1 and 2, at page 7, second and third paragraphs, the paragraph bridging pages 8 and 9, and at page 9, second paragraph. The amendment to claim 52 to recite "or" is supported, for example, by original claim 17, at page 7, second and third paragraphs, and the paragraph bridging pages 8 and 9. Thus, as the claim amendments are supported throughout the originally filed specification, no new matter has been added and entry thereof is respectfully requested.

The New Claims

Claims 55 to 59 are supported throughout the specification. In particular, claims 55 to 59 are supported, for example, by originally filed claims 1 and 2, at page 7, second and third

paragraphs, and at page 9, second paragraph. Thus, as claims 55 to 59 are supported throughout the originally filed specification, no new matter has been added and entry thereof is respectfully requested.

Exhibit C

Attached for the Examiner's consideration are selected pages from PCT WO 2010/088739 (a complete copy is submitted in the accompanying IDS). Applicants note that PCT WO 2010/088739 discloses, among other things, variant antibodies, antigen identities, and studies of variant antibody binding to target cells that express antigen to which SAM-6 antibody binds (Exhibit C, Examples 13-19). Notably, the attached include a description of binding studies demonstrating that SAM-6.10 (aka SAM-6) ScFv, sequence variants and a heavy chain variable region sequence alone (VH alone, SEQ ID NO:3) without a light chain variable region sequence, have binding activity for apolipoprotein B100, LDL, VLDL and/or deglycosylated LDL (Examples 13 and 19). In particular, ELISA analysis revealed that ScFv SAM-6, SAM-6 sequence variants and a heavy chain variable region sequence SAM-6 VH alone (SEQ ID NO:3) without any light chain variable region sequence binds to apolipoprotein B100 (Exhibit C, page 99). Accordingly, Applicants respectfully request consideration of the accompanying binding data.

Objections to the Figures

The Examiner has maintained that the Descriptions of Figures 1 and 2 allegedly are unclear, namely for lacking units for the y-axis label.

Applicants respectfully point out that the data in Figures 1 and 2 would be understood by one of skill in the art in view of the description of the two respective figures in the specification at pages 19-20.

In particular, in the paragraph bridging pages 19-20, the description states that "Figure 1 shows the measurement of oxLDL in dependence on the incubation time," and that "in the experiment, LDL....was oxidized for 3 and 15 h," and that "the amount of oxidized LDL increases with increasing incubation time." The y-axis of both Figures 1 and 2 show increasing values of "0.2," "0.4," etc. Consequently, the y-axis obviously reflects the LDL oxidation, which increases with increasing incubation time as reflected by the longer bars and as described in the Figure descriptions. Thus, the amount of LDL and units are not necessary to understand that more oxididized LDL is present (again as reflected by the longer bars) with

increasing oxidation time from 0, to 3 to 15 h. Consequently, the data represented in Figure 1 showing increased LDL oxidation over the indicated time periods would be understood by one of skill in the art.

In terms of the assertion that "Figure 2 cannot be evaluated because there is no distinction between the kontrolle and SAM-6 as the bars are both open," at page 20, second paragraph, the description states that "Figure 2 shows the proof of binding of SAM-6 to oxLDL," and that "the result shows that the more LDL that is present in its oxidized form, the more strongly the antibody SAM-6 according to the invention binds," which is indicated by longer bars at each respective time point. At page 20, second paragraph, the description also states that "the ELISA plate was precoated with LDL fractions oxidized to different degrees," which as stated in the Figure 1 description is indicated by the 0, 3 and 15 h time points along the x-axis and the longer bars reflecting increased LDL oxidation. Consequently, obviously one of skill in the art based upon the foregoing description understands that the longer bars at each successive time point in Figure 2 reflect SAM-6 binding (i.e., "the more LDL that is present in its oxidized form, the more strongly the antibody SAM-6 according to the invention binds"), and the shorter bars in which there is little apparent change reflect control (i.e., Kontrolle). Consequently, the data represented in Figure 2 would be clear to one of skill in the art.

Furthermore, the Examiner requests submission of new drawings "with the axes properly labeled and unites clearly identified for each figure." However, as much as Applicants would like to comply, the submission of such new figures would likely constitute addition of new matter, particularly since the Examiner believes that the figures are deficient and the submission of the proposed revised figures would require additional information in order to rectify the alleged deficiencies. For this reason alone, Applicants respectfully cannot submit new drawings as requested by the Examiner since doing so would potentially introduce new matter.

In any event, the data represented by Figures 1 and 2 would generally be understood by one of skill in the art: the Figures show relative differences that would be readily understood by one of skill in the art, and the USPTO does not require that Figures be represented in a particular format, let alone illustrate units or that a scale or statistical information be included. Moreover, Figures 1 and 2 are not essential to a technical understanding of the invention or to evaluate patentability of the claims.

In view of the foregoing remarks, the data represented by Figures 1 and 2 in combination with the description of the data in the as-filed specification would be sufficiently clear to one of skill in the art. Consequently, Applicants respectfully request that the objection to the specification be withdrawn.

Objection to the Substitute Sequence Listing and Specification

The Substitute Sequence Listing and amendment to the Specification previously submitted to correct errors in the sequences stands objected to due to the addition of allegedly new matter, namely the correction of 2 incorrect amino acid residues in SEQ ID NO:3 from Lys-Thr to Arg-Pro. Allegedly, there is no written description to support the amendment to correct the sequence error

Applicants respectfully point out that this issue was squarely and unambiguously addressed by the court in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002). In particular, the court held "that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material," and that "reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement." Id. at 965 and 970. In view of the fact that deposited sequences satisfy the written description requirement for those sequences, the referenced deposited biological material (DSM ACC2903) in the specification adequately describes the recited heavy (SEQ ID NO:3) and light chain (SEQ ID NO:1) variable region sequences. Accordingly, the previously filed Substitute Sequence Listing and amendment to the Specification correcting the sequence error of SEQ ID NO:3 does not add new matter, and the grounds for objection must be withdrawn.

The specification also stands objected to due to alleged defects in the previously filed Statement under 37 C.F.R. §1.804(b). Submitted herewith is a Revised Statement under 37 C.F.R. §1.804(b) executed by Dr. Frank Hensel that makes a specific reference to the hybridoma that produces SAM-6.10 antibody referenced in the application (for example, at page 13, lines 8-9). Accordingly, Applicants believe that the Revised Statement remedies the alleged defects in the previously filed Statement under 37 C.F.R. §1.804(b), and respectfully request withdrawal of the grounds for objection.

I. REJECTION UNDER 35 U.S.C. §112, FIRST PARAGRAPH ENABLEMENT

The rejection of claims 27 to 32, 34 to 43 and 48 to 51 under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement is respectfully traversed. The grounds for rejection are set forth in the Office Action, pages 6-14.

Applicants respectfully point out that claims 27 to 32, 34 to 43 and 48 to 51 require both light (V_L) and heavy (V_H) chain variable region sequences. Thus, given that these claims require both a light chain (V_L) variable region and a heavy chain (V_H) variable region sequences, all 6 CDRs that confer antigen binding activity are present. Furthermore, certain dependent and independent claims recite the predicted CDRs of heavy (V_H) and/or light (V_L) chain variable region sequences of SEQ ID NOs:1 and 3, which confer antigen binding specificity.

Moreover, as disclosed in previously submitted Exhibit B and corroborated by Exhibit C submitted herewith, heavy chain (V_H) variable region alone, without light (V_L) is sufficient to confer binding to target. Thus, given that all claims include a heavy (V_H) chain variable region sequence which alone is sufficient to confer binding activity, clearly antibodies and polypeptides including a variant heavy chain (V_H) variable region sequence within the scope of the claims could be readily identified without undue experimentation by routine binding studies disclosed in the specification or known to the skilled artisan.

To reiterate, as previously pointed out in the record the guidance in the specification and the high level of knowledge and skill in the art at the time of the invention relevant to antibody structure and function allows variants that bind LDL or oxLDL to be produced and identified without undue experimentation. Here, the sequence that confers binding to LDL or oxLDL is disclosed (SEQ ID NO:3). Consequently, variants of SEQ ID NO:3 could be readily produced and screened for binding to LDL or oxLDL using routine methods.

Applicants previously described an example of identifying binding variants. Namely, in view of the high level of knowledge concerning antibody structure correlating with function as acknowledged in a prior Office Action and disclosed in the specification, and the fact that SEQ ID NOs:1 and 3 and their predicted CDRs are disclosed, the skilled artisan would therefore be able to intelligently modify sequences to produce variant antibodies by introducing selected changes to SEQ ID NOs:1 or 3, and then verify which antibodies bind to LDL or oxLDL, for example. Such a methodology of introducing changes into a heavy or light chain variable region sequence of an antibody known to bind to LDL or oxLDL and

identifying those that bind to LDL and/or oxLDL could be performed without undue experimentation at the time of the invention.

To corroborate Applicant's position, submitted herewith are pages (Exhibit C) from PCT WO 2010/088739, which provides a concrete example of identifying variant antibodies without use of any greater knowledge of antigen identity than is disclosed in the as-filed application. Applicants note that PCT WO 2010/088739 discloses, among other things, variant antibodies, and studies of variant antibody and heavy chain variable region sequence alone binding to apoB-100, VLDL, LDL, or de-glycosylated LDL. Applicants reference these specific studies from the published application as merely examples of studies that corroborate that additional knowledge of antigen identity greater than disclosed in the subject application is not essential to identify antibodies including variants that bind to LDL or oxLDL.

Significantly, the studies in Example 13 (pages 97-99) are an analysis of 4 different sequences of SAM-6 antibody (pages 87-91 of PCT WO 2010/088739 have the sequences of the variants) and heavy chain variable region sequence alone for binding to apoB-100, VLDL, LDL, and/or de-glycosylated LDL. Using conventional ELISA methods, variant antibody and heavy chain variable region sequence alone binding to apoB-100, VLDL, LDL, or de-glycosylated LDL was determined. The foregoing study is one exemplary method by which one of skill in the art could identify variant antibodies without any more knowledge of antigen or epitope identity than that which is disclosed in the as-filed application.

In addition to Example 13, the data in Example 19 (page 102) refers to a particular SAM-6 variant, which exhibited increased binding affinity for LDL. Again, binding of the variant antibody to LDL was determined without the need for additional knowledge concerning the identity of the target antigen or epitope of LDL or oxLDL.

In sum, the foregoing data convincingly demonstrate that additional knowledge greater than what is disclosed in the as-filed specification as to the identity of the target antigen or epitope in LDL or oxLDL to which the claimed antibodies bind is not essential for one of skill in the art to identify variants within the scope of the claims. Consequently, the studies corroborate that one of skill in the art could identify variants of SEQ ID NOs:1 and 3 that bind to LDL or oxLDL without undue experimentation.

Lastly, in regard to the assertion in the Office Action (page 12) that it is unclear what is the target epitope and that there are differences in lipid compositions among LDLs, as is well understood in the immunology art, antibodies typically bind to proteins due to their

strong immunogenicity, and proteins are more likely to be immunogenic than lipids. Furthermore, it is well understood in the art that there are very few proteins present in LDL and oxLDL, and that LDL and oxLDL share a very limited number of proteins between them. Such proteins include Apoprotein B 100 (apoB). In fact, as stated in the Terrlink et al. publication cited by the Examiner (J. Lipid Research 45:954 (2004)): "each LDL particle contains a single copy of apolipoprotein B-100" (page 955, first column) Thus, given the fact that 1) proteins are known to be more immunogenic than lipids; and 2) there is only a single apo-B-100 in LDL, which is shared with other forms of LDL including oxLDL, clearly the number of possible epitopes to which the claimed antibodies could bind is far fewer than the Patent Office suggests. Moreover, the studies in previously submitted Exhibit B and Exhibit C submitted herewith clearly demonstrate that one of skill in the art could identify variants of SEQ ID NOs:1 and 3 that bind to LDL or oxLDL without any additional knowledge of antigen or epitope identity than that which is disclosed in the as-filed specification.

In sum, variants of SEQ ID NOs:1 and 3 that bind to LDL or oxLDL within the scope of the claims could be readily identified without any additional knowledge of antigen or epitope identity than that which is disclosed in the as-filed specification without undue experimentation, as corroborated by Exhibits B and C. Accordingly, the claims are adequately enabled under 35 U.S.C. §112, first paragraph, and the rejection must be withdrawn.

WRITTEN DESCRIPTION

The new grounds for rejection of claims 43 and 52 to 54 under 35 U.S.C. §112, first paragraph as allegedly lacking an adequate written description is respectfully traversed. According to the Patent Office, allegedly the correction of 2 incorrect amino acid residues in SEQ ID NO:3 from Lys-Thr to Arg-Pro is new matter.

As discussed above, the court in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956 (Fed. Cir. 2002) held "that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material," and that "reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement." Id. at 965 and 970. In view of the fact that a deposit of sequences satisfies the written description requirement for those sequences, the referenced deposited biological material (DSM ACC2903) in the

specification adequately describes the recited heavy (SEQ ID NO:3) and light chain (SEQ ID NO:1) variable region sequences. Accordingly, the amendment correcting the sequence error of SEQ ID NO:3 from Lys-Thr to Arg-Pro does not add new matter, and the rejection under 35 U.S.C. §112, first paragraph, must be withdrawn.

II. DOUBLE PATENTING REJECTION

The provisional rejection of claims 27 to 32, 34 to 42 and 48 to 54 under 35 U.S.C. §102(b), as allegedly unpatentable over claims 73, 80, 81, 106 to 112, 115, 116 and 122 to 124 of co-pending application no. 10/579,290 is respectfully traversed. Allegedly, claims 27 to 32, 34 to 42 and 48 to 54 would have been obvious in view of the conflicting claims of application no. 10/579,290.

Applicants respectfully request that the rejection be held in abeyance until such time as all other rejections are withdrawn. Applicants will at that time file a remarks traversing the rejection or a Terminal Disclaimer, as appropriate.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 27 to 32, 34 to 43 and 48 to 59 clearly and patentably define the invention, and respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

For purposes of the Interview, the Examiner is respectfully requested to contact Applicant's representative at (858) 509-4065.

Please charge any fees associated with the submission of this paper to Deposit Account Number 033975. The Commissioner for Patents is also authorized to credit any over payments to the above-referenced Deposit Account.

Respectfully submitted,

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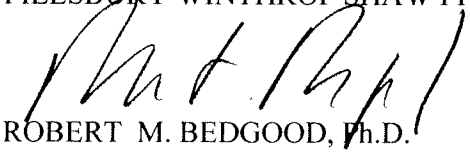

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included, an LM1 diabody, CM1 diabody, a control VH dimer-which is actually a monomer, a BARB3-diabody, BARB4 diabody, a recombinant PAT-SAM6 450-IgM (produced by PerC6 cells, Percivia), recombinant LM1 41B1-IgM , and SAM6 C8/9 hybridoma IgM. The negative controls are the conditioned media that the A549 cells have been growing in, without the primary antibody and with the secondary antibody. The data show that all of the SAM-6 diabodies bind to an antigen present in the A549 culture supernatent, it is also detected by the recombinant SAM-6 IgM clone 450 (produced by PerC6 cells, Percivia). SAM-6 hybridoma C8/9 gives a very poor signal, but this may be due to protein degradation or hybridoma cell death. This ELISA also shows that the other antibodies tested do not bind to any secreted product in the A549 conditioned media. Only SAM-6 detectably binds to the A549 conditioned media. This ELISA shows that SAM-6 binds to a target in A549 cell conditioned media.

Conditioned media from a second cell line HDFa previously shown not to exhibit cell surface binding to SAM-6 antibodies was studied for binding to SAM-6 antibodies. No binding was detected indicating that this cell line is a good negative control.

Another ELISA was performed on a plate coated with Grp78 protein (from Abnova- cell free protein translation-using wheat germ-non-glycosylated). Binding of recombinant PAT-SAM-6 IgM antibody (clones 450 and 528 produced by PerC6 cells, Percivia) and recombinant SAM-6 1.1A diabody to pure non-glycosylated Grp78 protein was detected. All binding was to pure (non-glycosylated) target Grp78 protein. This data indicates that SAM-6 antibodies and variants bind to grp78 without a carbohydrate moiety. On the second half of the plate binding to conditioned media from A549 cells was detected, whereas negative control LM1 antibodies did not bind to the conditioned media. There was variation in the strength of the signal detected but the target protein may not be uniformly dispersed throughout the sample.

Example 13

This example includes a description of studies showing various forms of SAM-6, including SAM-6 scFv, SAM-6 variants and SAM-6 heavy chain variable region (V_H) alone, without light chain variable region (V_L) bind to an apoB100, protein, LDL, VLDL and deglycosylated LDL.

Antigen specificity: Fresh batches of recombinant protein were made and tested against a panel of proteins to determine specificity for LDL. The ELISAs were repeated several times. Positions of the antigens on the plates were randomized to rule out position effects.

For SAM-6 KTA scFv, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 KTA scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). Note that in these protein samples the soluble B fraction has been removed and only the remaining soluble C fraction is tested. The 3rd time reading are higher as they contain the combined protein level (1CHO4.8) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49). The well contains the antigen, Lewis Y tetrasaccharide bound to HSA(Human Serum Albumin). The positive control (anti-Lewis Y) gave an absorbance reading at A655nm of 0.98 on one ELISA and 0.90 on the other ELISA when binding to its carbohydrate antigen lewis Y.

The strongest binding of SAM-6 KTA scFv is to Apolipoprotein B100. Binding to VLDL, LDL and deglycosylated LDL was also detected.

For SAM-6 1.1A scFv urea solubilized, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 1.1A scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). The 3rd time readings contain the combined protein level (1CHO4.7) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49) gave an absorbance reading at A655nm of 1.2 and 1.0.

Strong binding of SAM-6 1.1A to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 (Percivia), strong binding to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 HAB produced by human hybridoma (Patrys GmBH, Germany), strong binding to VLDL, LDL and de-glycosylated LDL, was detected but less binding to apolipoprotein B100. The SAM-6 HAB gave variable results in this assay.

In the foregoing studies several different SAM-6 proteins produced in a variety of different formats were compared for their ability to bind to various target antigens, such as LDL (Low Density lipoprotein), VLDL, deglycosylated LDL and apoB100 protein. SAM-6 KTA scFv, 1.1 scFv, PAT-SAM-6 (Percivia) and SAM-6 HAB exhibited various degrees of binding affinity for LDL, VLDL, deglycosylated LDL and apoB100 protein, but not HDL (high density lipoprotein). In this way sequence changes can be linked to function.

Further binding studies to ApoB100 were performed by ELISA analysis. In brief, 250ul of Apolipoprotein B100 (10ug/ml) isolated from low density LDL (purchased from Calbiochem) was coated onto ELISA plates. Plates were blocked, incubated with primary single-chain antibodies (SAM-6.2.7 and SAM-6.opti) and SAM-6 heavy chain variable region (V_H) alone), and then incubated with anti-FLAG-HRP secondary antibody in a total volume of 250ul, and compared to three negative controls (Negative control 1: No coating (blocked), No primary, then anti-FLAG-HRP secondary; Negative control 2: No coating (blocked), then primary, then anti-FLAG-HRP secondary; and Negative control 3: Coated with 10ug/ml ApoB100 (blocked), No primary, then anti-FLAG-HRP secondary).

The results indicated that SAM-6.2.7, SAM-6.opti and SAM-6 heavy chain variable region (V_H) alone) bind to ApoB100 protein.

Example 14

This example includes a description of studies showing that SAM-6 variants can also bind to cancer cell lines A549, BxPC3 and CRL1424.

The following variants were studied: SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia). SAM-6 VHVL opt scFv has an optimized framework with 4 amino acid changes in the VH domain including 25% changes at the nucleotide level. There is one additional change in CDR-H1. The VL domain of SAM-6 VHVL opt scFv is a class switch from lambda to kappa light chain with 40 amino acids changed including 38% changes at the nucleotide level. The free Cys residue was removed from the VL CDR1.

FACS analysis revealed that all of the scFv constructs bind to the three cancer cell lines tested (A549, BxPC3 and CRL1424), but not to the negative cell line HDFa.

Additional studies were performed using confocal microscopy analysis for binding to A549, BxPC3, CRL1424, HT-29, HeLa, and MCF-7 cancer cell lines. The SAM-6 antibodies studied included SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia).

In brief, cells were fixed, the primary antibody was added, then detected with a secondary antibody with a FITC label. The cell nucleus was stained with a DAPI stain that appears blue, and measured in the 600-650 wavelength range. This DAPI image was captured and recorded. If the level of DAPI nuclear stain was kept at a constant level, different studies can be "normalized." The cells were incubated with the primary (test) antibody, and the appropriate labelled secondary

PAT-SAM6-IgM shows binding to LDL relative to isotype matched human IgM antibody (Figure 25A). Furthermore, binding of PAT-SAM6-IgM is increased after Cu2+ oxidation of LDL (Figure 25A). Antibody-induced lipoptosis of tumor cells in the presence of differently Cu-oxidized LDL was measured by Cell Death Detection ELISAPLUS. Pancreatic carcinoma cell line BXPC-3 was incubated with PAT-SAM6-IgM and unrelated human IgM isotype control. Amounts of apoptotic cells were determined photospectrometrically at 415 nm (reference λ 490 nm). PAT-SAM6-IgM ability to induce lipoptosis/apoptosis is enhanced in the presence of increased Cu^{2+} oxidized LDL (Figure 25B).

Example 18

This example includes a description of studies showing SAM-6 immunoprecipitation of target antigen, and possibly antigens associated with target antigen, from conditioned media produced by A549 cells.

Immunoprecipitation studies of conditioned media from A549 cells with SAM-6 diabody was performed. The immunoprecipitated portion was fractionated on a10% SDS-PAGE and subsequentely silver stained. As illustrated in Figure 26, SAM-6 diabody binds to several proteins around 110 to 50 kDa, as well as lower molecular weight proteins, present in the A549 cell conditioned media. These proteins may be a SAM-6 target, a target fragment or a protein that is associated with a SAM-6 target. The 30 kDa is presumed to be the SAM-6 diabody.

Example 19

This example includes a description of additional studies showing increased affinity of a particular SAM-6 variant (optimized scFv dimer) for LDL, as compared to SAM-6 1.1A scFv and SAM-6 optimized scFv monomer.

PAT-SAM-6-IgM was previously been shown to bind LDL and induce lipoptosis in cancer cells. Affinity of other PAT-SAM-6 scFv variants to LDL was assessed via ELISA. All variants bind positively to LDL, with PAT-SAM-6 scFv diabody optimized having the greatest affinity.

Example 20

This example includes a description of additional SAM-6 binding studies, and that SAM-6 does not bind to CD55 antigen.

In brief, CD55 antigen (0.5 µg/well) was coated in a volume of 50µl/well. The primary antibody was incubated at 12 µg/ml (0.6 µg/well). Buffer was pH6.5 and for the dilution buffer used was High salt pH8.0. These ELISA studies revealed that SAM-6 does not bind to CD55 antigen.